

Bio-Inspired Materials and Devices for Chemical and Biological Defense

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Introduction

Living systems are capable of manufacturing processes, molecular recognition and other complex functions which cannot be replicated by synthetic chemistry or other industrial technologies. Cells routinely manufacture monodisperse nanoscale structures and assemble molecular machines, carry out biochemical reactions and production processes of great complexity, and interact with the environment in an adaptive and emergent manner. Biotic (i.e., living) systems can be labile and, by their nature, difficult to precisely control. The ability to elucidate key metabolic pathways and to replicate their functional properties in a synthetic (i.e., abiotic) format will ultimately permit the design of completely artificial systems with abilities similar to those of a biotic system but with the advantages of precise process control and enhanced ruggedness. This will have profound implications for the many and varied missions of the Department of Defense (DOD) which include, but are not limited to, small-scale power and energy, lightweight flexible armor, on-demand manufacture of high-value products such as pharmaceuticals, low observable materials and—the subject of this paper—chemical and biological defense (CBD).

Transformational advances in CBD are expected to depend heavily on biologically inspired technology. A leading vision in this direction is a conceptual platform known as the abiotic networked threat system (ANTS). ANTS is based on lessons learned from biology, incorporating abiotic homologues to biological recognition events and metabolic pathways to provide programmable capabilities to sense and respond to environmental threats. Further, it is a complete platform architecture with intelligent preprocessing and network capability and is envisioned to be embedded in all aspects of CBD including detection, communication, activation of response systems, and protection systems. The six major components in ANTS are:

1. Molecular recognition elements for xenobiotic threats
2. Materials for synthetic matrices
3. Signal transduction in biotic, hybrid and synthetic systems
4. Power generation at the nanoscale
5. Computational tools for design of abiotic units, devices and modules
6. Combinatorial design and deployment of synthetic systems

On a very simplified level, the objective is a collection of self-sustaining micro- or meso-scale devices that mimic the xenobiotic (i.e., from outside the cell or tissue) threat recognition, response and adaptation of living cells. These units would operate as a decentralized network, communicating with one another, processing information, and triggering responses which may exhibit emergent properties.

Recognition elements, power, signal transduction, and networking are generally recognized as essential and challenging areas for research in this concept. Less obvious are the challenges associated with the physical support matrix required to support and shelter the units. This report addresses area 2, materials for synthetic matrices.

Background

The problem of materials selection and development for membranes and support structures cannot be addressed independent of the problems of recognition, transduction, amplification, and communication. In fact, the materials need to interface with, support, and complement the functional aspects of the platform as a whole. As such, the approach to the materials problem should be agile and holistic, addressing materials for future technology while providing appropriate materials for intermediary technologies.

In the natural living cell, the membranes and support structures (e.g., microtubules) are integral parts of the functional activities of the cell, and it could be argued that the inverse is equally valid: that the many functional activities of the cell are integral parts of the membrane. Proteins and lipids in the membrane are responsible for transporting nutrients, products, and information through and across the membrane, and the biomolecules involved in these processes are usually constituents of the membrane. The extent and nature of this interdependence varies with the specifics of the biochemical pathway in question. Thus, the role of membrane materials in abiotic cell homologues depends largely on the biochemical pathways being simulated and on the abiotic simulation technology being addressed. By extension, the type of support and interaction required from the membranes will be dictated by the nature and maturity of the pathway simulations. This gives four scenarios of environmental or mobility dependence that will affect membrane development, summarized below. In Table 1, a fifth scenario is included in which the functional elements are so environmentally stable, and their function is so elegantly engineered, that they require no protection or mobility control from the membrane. This attractive, though unlikely, scenario is the only one in which the membrane material serves a purely support function. Research toward the realization of an abiotic threat sensor platform and its integration into myriad CBD equipment and systems can be expected to progress through these scenarios, with different material technologies required at each step. What follows is a discussion of existing and emerging material technologies that will be relevant as such a concept progresses from initial investigations to the long-term vision.

1. The functional components are subject to the same or equivalent environmental requirements as their biological homologues and require the same or equivalent through-membrane and intra-cellular mobility.
2. The functional components are not dependant on environment but do require through-membrane and intra-cellular mobility.
3. The functional components do not depend on the environment nor do they require through-membrane mobility, but they do require intra-cellular mobility.
4. The functional components do not depend on the environment nor do they depend on intra-cellular mobility, but they do require through-membrane mobility.

Table 1. Possible scenarios for environmental and functional element mobility requirements to be faced by support materials

	Environmental Dependence	Functional Element Mobility Dependence	
		Intra-cellular	Cross-membrane
1	Y	Y	Y
2	N	Y	Y
3	N	Y	N
4	N	N	Y
5	N	N	N

In the early research stages, the units are conceptual and research is aimed at making the functional components, that is, the recognition elements, transduction pathways, and homologues of associated biochemistry function. This constitutes a bread-board or bench-top capability, with a focus on:

1. Understanding and identifying the functional components to be synthesized
2. Recreating the components external to living cells
3. Synthesizing abiotic homologues for the functional components
4. Understanding and identifying the interactions between the components and between the components and environment
5. Recreating these interactions

Functional components would be investigated separately and in discrete combinations to establish proof-of-principle and for optimization. At this stage, materials requirements would be associated with localizing, immobilizing, and stabilizing individual elements, as well as possible facilitation of interoperability of components.

To facilitate these early developments, support materials need to provide means for any environmental interaction required. Ideally, any requisite environmental protection would also be provided, though this may not be necessary for all intermediate proof-of-principle prototypes. This is akin to situation 1 from Table 1; something very close to a natural cell membrane will be required. Clearly this is not a desired end state for a fieldable product. However, in the research stages it will be likely that one or more investigational functional element will have these requirements. As technologies targeted for functional elements move deeper into the abiotic realm, the levels of environmental and mobility requirements will shift.

As abiotic technologies advance toward the vision of independent and stable units that recreate the relevant actions of biological homologues (i.e., imitate living systems), the materials requirements will evolve toward more hardened materials and become more tailored to support specific technologies. Because the architecture for an ANTS platform will be technology driven rather than predetermined, the final form of the units could follow any one of numerous directions. In the closest homologue to the living cell, the units would be several functional components housed within a structure that allows the

components to move and interact in a programmable manner or in response to ambient conditions while also allowing one or more to traverse the enclosure and interact with the exterior environment. Some of the substrate and functional element interactions possible for viable ANTS products include:

1. Hardened material carrying functional components on its surface
2. Hardened material carrying functional components internally and providing interaction with the environment
3. Hardened material carrying some functional elements on both its surface and interior with communication facilitated by substrate
4. Functional material with other functional components embedded and interacting within the matrix
5. Functional material carrying some functional elements on both its surface and interior with communication facilitated by substrate which participates in function.

This presents a range of possibilities, each of which in turn presents unique challenges in material development. The simplest case of a hardened material, carrying surface elements would require surface properties amenable to functionalization with the elements and allow interactions among the elements. A hardened material with internal elements would require controllable permeability, pores, or through-channels to allow correct environmental interaction and possibly require the ability to precisely position and immobilize, or provide specific mobility to, functional elements. Hardened material with functional elements on both the interior and exterior would have the same requirements as a hardened material with only internal elements, but with the possibility of added positioning and immobilization requirements to allow correct relative positioning and interaction of internal and external moieties. Situations 4 and 5 would pose similar requirements to 2 and 3, with the added requirement of any specific positioning or motility required to facilitate the various functional activities.

Clearly, there is a broad range of possible material requirements that may be faced by functional abiotic aggregates. The extent of these requirements changes with the level of complexity of each functional element and of the interactions among the elements. As ANTS and similar technologies mature, these requirements will evolve, both from the perspective of increasingly complex functional interactions and from the decreasing dependence of functional elements on the environment.

The evolution of functional interactive elements will require the simultaneous evolution of matrix materials for support and facilitation of elements and element function. What follows is an overview of candidate materials that can be expected to play a role in the support, protection, and facilitation of functional elements in an abiotic platform.

Current Technologies

Starting with a purely biotic (i.e., living) system, the target material is the outer membrane of a living cell. Structurally, the prime constituents of the cell membrane are two layers of phospholipid molecules. These molecules align themselves such that polar head groups are in contact with the surrounding environment (extracellular) and with the interior of the cell, both aqueous environments, while the hydrophobic tails associate themselves with hydrophobic lipid tails from the facing layer. This establishes an arrangement of hydrophilic-hydrophobic-hydrophilic regions as you pass between the interior and exterior of the cell. The hydrophobic region effectively blocks unwanted movement of aqueous solution between the interior and exterior. A number of transmembrane structures and proteins exist to facilitate desired movement of material and information between the cytoplasm and extracellular fluid. Thus the structural material most closely resembling the natural cell is a lipid bilayer, either as a film or in the vesicle form of a liposome. Within the bilayer itself, rafts of phospholipids move laterally to relocate functional molecules and information. Within the lipid layers of a cell membrane, heterogeneities arise from immiscibility among various lipids. Segments known as detergent resistant membrane fractions (DRM), or rafts, have been found to be more tightly packed than the surrounding bilayer and to float freely within the membrane. This rafting is suspected to be instrumental in a variety of cell activity, including receptor signaling. As early as 1956, Thomas Chang was working on the first concepts of artificial cells¹. Even at that time, Chang recognized that the term “artificial cell” need not refer to a specific physical entity, let alone any given size or shape, but rather to the capability of a synthetic structure of approximately cellular size to carry out one or several activities normally attributed to cellular function. Early investigations in the area considered a wide range of materials such as cellulose nitrate, synthetic rubber, proteins, polysaccharides, lipids, and combinations, and were typically made via interfacial precipitation or interfacial polymerization. The goals of these early artificial cells were almost exclusively to function as a cell in environments typically inhabited by living cells, i.e., they were designed for use in aqueous media and for encapsulation of aqueous environments, and applications were primarily medical.

Artificial cell technology remains a very active research area in the medical arena. Applications range from therapy delivery and tissue engineering to blood substitutes and gene therapy. The list of realized and investigational applications is extensive.²

¹ Chang, T.M.S. *Artificial Cells*, Charles C. Thomas: Springfield, IL, 1972.

² *Artificial Cells, Cell Engineering and Therapy*. Prakash, S., Ed.; Woodhead Pub. Ltd., Cambridge, England, 2007.

Cell Membranes and Lipid Bilayers

In this approach, a lipid bilayer similar to the liposome layers or cell membrane is constructed across a small pore or opening much like a microscopic soap bubble film.³ These bilayers are used to study functional activities requiring biochemical activity that crosses the membrane. This could include either biological macromolecules that bridge through the membrane or functions that involve small molecules traversing the membrane. Lipid bilayers can also be supported on solid surfaces; however, this generally poses problems with bridging macromolecules that need to be free on both sides of the bilayer membrane. One solution to this involves suspending the bilayer on porous substrates.⁴ Successes in this area point to the possibility of linking surface roughness factors to functionality in suspended bilayers.

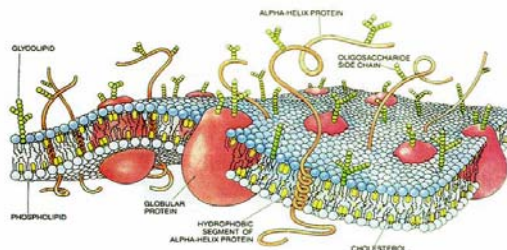


Figure 1. Conceptual drawing of cell membrane.

Studies into the function of cell membranes led scientists to develop extracellular lipid bilayer membranes. It is typical for these to be supported across a gap in an impermeable material and held between two aqueous solutions. In this way the transmembrane activity can be probed. Although these approaches have been known since the 1960s, use of such “black lipid membranes” (BLM) has expanded and become more sophisticated with the explosive growth of biotechnology and nanotechnology.

Lipid bilayers are often supported on solid nonporous surfaces for a variety of applications.⁵ In these cases, membranes are integrated with a layer covalently linked to the support, separated from the support by thin films of water or polymer, or cushioned by a film of hydrophobic molecules. Supported membranes are typically assembled on the substrate by monolayer transfer according to Langmuir-Blodgett techniques or by vesicle spreading. A wide range of methods for creation of supported lipid membranes have been reported and many are used for incorporation and subsequent studies of membrane proteins.⁶ These approaches generally retain the structural and thermodynamic properties of free bilayers and can be functionalized, allowing valuable studies on the properties and behavior of the

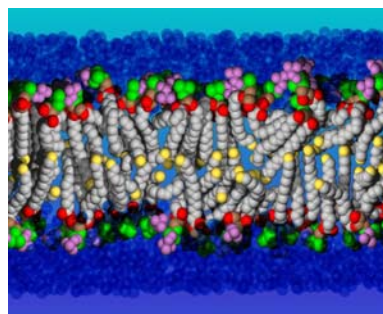


Figure 2. Lipid Bilayer

³ Ichikawa, T.; Aoki, T.; Takeuchi, Y.; Yanagida, T. and Ide, T., Immobilizing Single Lipid and Channel Molecules in Artificial Lipid Bilayers with Annexin A5, *Langmuir* (2006) 22, 6302-6307.

⁴ Worsfold, O., Voelcker, N.H., Nishiya, T., Biosensing Using Lipid Bilayers Suspended on Porous Silicon, *Langmuir* (2006)22, 7078-7083.

⁵ Sackmann, E. Supported Membranes: Scientific and Practical Applications. *Science* 1996, 271, pp 43-48.

⁶ Lindhom-Sethson, B. Supported Lipid Membranes for Reconstitution of Membrane Proteins. *Focus on Biotechnology* 2007, 7, pp 131-165.

film and functional elements. However, the space between the bilayer and the substrate is inadequate for transmembrane studies, which require a true free standing membrane.

To provide access to both sides of a bilayer, traditional BLMs are supported over openings on the order of 0.1 mm, which allows fluidity but has limited stability. At the Paul Scherer Institute in Switzerland, researchers are building nanochips for functional assays. Supporting lipid bilayers across nanopores in ultra thin SiN membranes and placing this composite film between two compartments allows cross-membrane activity while improving on the stability of the film.⁷ Other groups have developed alternate methods based on the same general concept. The Posner group at Arizona State University⁸ suspends lipid bilayers across 40 nm pores in silicon and inserts a protein ion channel to conduct current from one side of the chip to the other. Specific applications of this are in the area of single-molecule detection. Following a similar principle, investigators⁹ have inserted α -hemolysin nanopores into lipid bilayers such that the pore is positioned to pass through the membrane. These membranes, when placed between two salt solutions, allow current to pass through the nanopore. Attaching molecular recognition agents to the inner surface of the pore renders the pore selective, such that when an analyte binds with the agent the current is blocked for a length of time dictated by the chemistry of the binding event. This approach has been shown to be effective for selective sensing of metal ions, proteins, drugs, and oligonucleotides.

The lipid bilayer approach to transmembrane studies has become prevalent enough that analyzers and artificial bilipid membranes developed specifically for this purpose have become commercially available.¹⁰ In addition to studying transmembrane conduction, lipid bilayers have been used to study lateral diffusion of single molecule lipids and proteins in artificial bilayers, and methods of reducing the lateral diffusion by a factor of 200 with the use of annexin A5 have been reported.³ Another approach to the use of lipid bilayers involves arrays of supported bilayers, which are synthesized by stamping. An agarose hydrogel patterned for a desired array is “inked” with lipids and used to selectively place an array of spots of supported bilayers.¹¹ These stamps have been used in studies of membrane properties and in the screening of drug-membrane and protein-membrane interactions.¹²

Fully supported and pore-suspended bilayers provide a very useful approach to studying the individual activity of functional elements, but are severely limited by the fragility of the bilayers. The obvious next step is to stabilize the membranes either for more demanding studies or for applications.

⁷ <http://sb.web.psi.ch/projects/bionanotechnology.html> (accessed June 2008)

⁸ Bayley, H.; Cremer, P.S. Stochastic Sensors Inspired by Biology. *Nature* 2001, 413, pp 226-230.

⁹ Martin, C.R.; Siwy, Z.S. Learning Nature's Way: Biosensing with Synthetic Nanopores. *Science* 2007, 317, pp 331-332.

¹⁰ <http://www.membrasense.com> (accessed June 2007).

¹¹ Membrane Printing Made Easy. *Nature Chemical Biology* [Online] 2005, 1(6), p 323.

¹² Majd, S.; Mayer, M. Hydrogel Stamping of Arrays of Supported Lipid Bilayers with Various Lipid Compositions for the Screening of Drug-Membrane and Protein-Membrane Interactions. *Angew. Chem. Int. Ed.* 2005, 117(41), pp 6855-6858.

Stabilized Lipid Layers and Bilayers

In order to put lipid bilayers to practical use, it is often desirable to stabilize the bilayer. Several approaches to this have been reported, some based on substrate support, on anchoring, or on chemical modification. In a number of applications, usually associated with surfaces to be used in contact with blood for medical purposes, a bilayer is not necessary and the desired product is a single, properly oriented layer of phospholipid.

In the preceding section, BLM spanning nanopores in silicon and silicon nitride membranes were discussed. This approach provides significant improvement in stability over BLMs supported over larger (~0.1 mm) pores.⁷ A number of other approaches to supporting and stabilizing lipid layers and bilayers have been reported. Continuing with support on nanoporous substrates, lipid bilayers have been successfully suspended on porous silicon and used to demonstrate single cell biodetection.⁴ The approach uses oxidized aminosilanized porous silica which is further functionalized by biotin, which allows for a biotin-streptavidin layer that distances the bilayer from the substrate, helping to maintain fluidity as well as providing access to the bottom of the bilayer. In this case, fluidity is required for lateral mobility of peptides.

Lipid bilayers are supported on nonporous substrates by a variety of methods.⁵ Typical approaches involve tethering the bilayer to a substrate using lipids either alone or in combination with more complex chemistries. Early supported lipid bilayers were put on substrates by simple physical adsorption, examples being vesicle fusion and Langmuir-Blodgett techniques. In vesicle fusion, a substrate is immersed in an aqueous solution of lipid vesicles. When vesicles contact the substrate surface they adhere, break and spread to form a lipid monolayer, in the case of hydrophobic surfaces, or bilayer, in the case of hydrophilic surfaces. Langmuir-Blodgett techniques involve spreading solutions of lipids in volatile organics across an air/water interface and transferring the lipid layer to a substrate after the solvent evaporates.

More sophisticated versions of these methods are often aimed at patterning the bilayer. Urisu *et al*¹³ looked at the formation of lipid membranes on surfaces modified with monolayer islands. Placing self-assembled monolayer islands of octadecyltrichlorosilane (OTS) on silica surfaces and using these surfaces for subsequent vesicle fusion techniques showed a dependence on the relative size of the vesicles and the monolayer islands. With dipalmitoylphosphatidylcholine (DPPC) vesicles, silica domains larger than the vesicles were covered by bilayers of DPPC while the OTS domains formed DPPC/OTS bilayers. Vesicles larger than the silica domains formed only DPPC/OTS bilayers with no DPPC bilayers on the silica.

As work in the area of supported bilayers becomes more complex, these physical approaches are being joined by a wide range of chemical tethering approaches. Gilchrist

¹³ Tero, R.; Takizawa, M.; Li, Y.-J.; Yamazaki, M.; Urisu, T. Lipid Membrane Formation by Vesicle Fusion on Silicon Dioxide Surfaces Modified With Alkyl Self-Assembled Monolayer Islands. *Langmuir* 2004, 20, pp 7526-7531.

*et al*¹⁴ have used biotin-PEG₃₄₀₀-bacteriorhodopsin conjugates to tether lipid bilayers to streptavidin-coated microspheres, providing more stability and control of the membranes and versatility in the attachment of membrane proteins.

Phospholipid polymers have also been successfully produced and employed. Amphiphilic phospholipid polymers have been applied to gold electrodes and used to immobilize antibodies. The active ester groups of the polymer are very effective in immobilization of biomolecules, and the attached antibodies have been shown to remain stable for 60 days.¹⁵ The same group¹⁶ developed phosphorylcholine surface technology (PCST) for assembling phospholipids and phospholipid polymers. Applied to material surfaces, this places an artificial cell membrane surface on a material, an approach which improves biocompatibility of surfaces and is generally useful for biointerfaces.

In a number of cytomimetic (cell mimics) approaches, a phospholipid monolayer is desired on the substrate. Some of the simplest approaches to this include immobilization by poly(ethylene glycol) (PEG), cholesterol mixing, chemical anchoring and chemical anchoring to a PEG-covered surface,¹⁷ though stability of these layers is often an issue. One approach to addressing stability is *in situ* polymerization of the lipid layer. Initial work in this area used thermal initiation of polymerization on alkylated substrates, though this precludes applications that incorporate thermally sensitive molecules in the lipid layer. More recently^{18,19,20} the same *in situ* polymerization processes have been realized using photo-initiation. In this series of studies, Chaikof *et al* used self-assembly of acrylate-functionalized phospholipid on alkylated glass and silica supports and the resulting layer was photo polymerized *in situ* via irradiation of eosin Y/triethanolamine. Resultant poly(phosphatidylcholine) was more stable than products of thermally initiated polymerization and showed stability in air, water and flow conditions. In order to improve stability of polymerized lipid layers in surfactant and organic solvents, Byun *et al*²¹ polymerized PEG-covered phospholipid monolayers on methacroyl-terminated surfaces of silanized Si wafers. In general, these monolayer approaches are suitable for medical applications in which it is desirable for the material to be compatible with blood, yet not exhibit unwanted adherence to proteins and cells.

¹⁴ Sharma, M.k.; Jattani, H.; Gilchrist, M.L.Jr. Bacteriorhodopsin Conjugates as Anchors for Supported Membranes. *Bioconjugate Chem.* 2004, 15, pp 942-947.

¹⁵ Park, J.; Kurosawa, S.; Takai, M.; Ishihara, K. Antibody Immobilization to Phospholipid Polymer layer on Gold Substrate of Quartz Crystal Microbalance Immunosensor. *Colloids Surf. B Biointerfaces* 2007, 55(2), pp 164-172.

¹⁶ <http://aiche.confex.com/aiche/2006/techprogram/P51540.HTM> (accessed June 2008)

¹⁷ <http://lcmt.snu.ac.kr/research/research4.php> (accessed May 2008)

¹⁸ Orban, J.M.; Faucher, K.M.; Dluhy, R.A.; Chaikof, E.L. Cytomimetic Biomaterials 4. In situ Photopolymerization of Phospholipids on an Alkylated Surface. *Macromol.* 2000, 33(11), pp 4205-4212.

¹⁹ Marra, K.C.; Winger, J.M.; Hanson, S.R.; Chaikof, E.L. Cytomimetic Biomaterials I. In situ Polymerization of Phospholipids on an Alkylated Surface. *Macromol.* 1997, 30, pp 6483-6488.

²⁰ Marra, K.G.; Kidani, D.D.A.; Chaikof, E.L. Cytomimetic Biomaterials 2. In situ Polymerization of Phospholipids on a Polymer Surface. *Langmuir* 1997, 13, pp 5697-5701.

²¹ Kim, K.; Shin, K.; Kim, H.; Kim, C.; Byner, Y. In situ Photopolymerization of a Polymerizable Poly(ethylene glycol) Covered Phospholipid Monolayer on a Methacroyl -Terminated Substrate. *Langmuir* 2004, 20, pp 5396-5402.

Although lipid layers and bilayers provide excellent mimicry of cell membranes and offer a range of parametric control, they are not expected to develop to a level of hardness and stability of use in practical field applications. They are, however, well-characterized systems that are very useful in the study of biological or biologically inspired activity, and are good choices for early studies on the various functional elements of abiotic materials.

Liposomes

In the early stages of development, membranes and support materials would look to optimize existing technologies to provide substrates or material environments in which research on functional components can be conducted. By far the most common approach to this is the liposome, typically a spherical lipid bilayer consisting of phospholipid and cholesterol layers which serve to isolate the interior of the “cell” from the exterior while allowing some molecular interchange between the interior of the “cell” and its environment. In general terms this could be considered a double layer micelle (micelles are single layer vesicles of amphiphilic molecules). Typically a micelle aligns hydrophilic head groups out toward an aqueous environment and entraps nonpolar materials inside the cell. The reverse situation, in which nonpolar tail groups align toward a nonpolar environment trapping polar materials inside, also occurs. In a liposome, polar head groups align both inward and outward, creating a hydrophobic barrier within the wall of the vesicle which separates two different aqueous environments, one inside and one outside the liposome. This is the closest available homolog to the membrane of a living cell. Liposomes have been heavily investigated by the drug delivery community as vesicles for encapsulating and delivering therapeutic agents and are used in early nanopharmaceutical products. There is therefore a rich literature background covering encapsulation and release of drugs using liposomes.^{22,23}

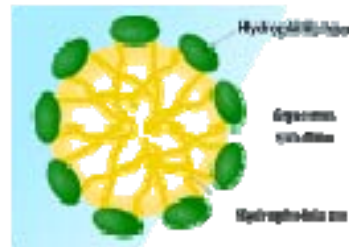


Figure 3. Micelle

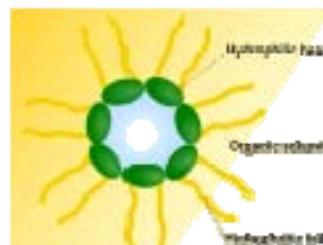


Figure 4. Reverse micelle.

Going beyond clinical applications, investigators have been studying phospholipid vesicles as bioreactors to act as artificial cells for protein expression. Moving protein expression from bulk solution to bilayer vesicles and isolating the transcription-translation of plasmid genes extends expression by more than a factor of two. However, the expression is limited by the energy and material limitations imposed by the vesicle. Noireaux and Libchaber²⁴ addressed this by expressing the α -hemolysin pore protein in

²² Schifflers, R.; Storm, G.; Bakker-Woudenberg, I., Liposome-encapsulated Aminoglycosides in Pre-clinical and Clinical Studies, *Journal of Antimicrobial Chemotherapy* (2001) 48, 333-344.

²³ Vemuri, S.; Rhodes, C.T. Preparation and Characterization of Liposomes as Therapeutic Delivery Systems: A Review. *Pharmaceutica Acta Helveticae* (1995) 70, 95-111.

²⁴ Noireaux, V.; Libchaber, A. A Vesicle Bioreactor as a Step Toward an Artificial Cell Assembly. *PAS* 2004, 51, pp 17669-17674.

the vesicle, providing permeability and allowing nutrients to enter the vesicle. These vesicles extended expression to as long as four days, opening the door to viable cell-free bioactivity. Eventually, this approach could lead the way to complete pathways encoded by “minigenomes” expressed without living cells. In an alternate approach Monnard²⁵ capitalized on the fact that, at temperatures approaching the melting temperature of the phospholipids, natural defects arise in the bilayer. Similar to the α -hemolysin approach this provides pores that allow ADP and ATP exchange while retaining DNA within the vesicle. Ha *et al*²⁶ used these techniques to study single molecule activity within vesicles tethered to quartz slides via a PEG-neutravidin-biotinylated lipid immobilization scheme.

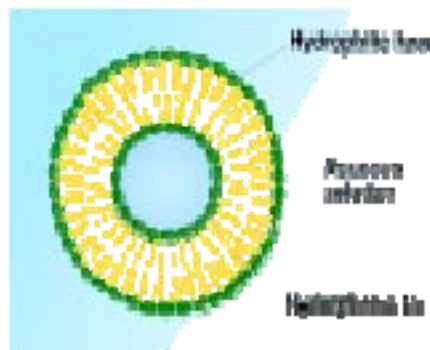


Figure 5. Lipid Bilayer Vesicle

Liposomes are fragile, environmentally unstable, generally used and studied in an aqueous suspension, and susceptible to coalescence. As a practical matter, this makes them unsuitable as stand-alone abiotic cell membranes but useful for functional studies and potentially useful as subunits in otherwise stabilized abiotics.

As with lipid bilayers, there is great interest in stabilizing liposomes and several approaches toward this end have been considered. Liposomes in suspension have a tendency to fuse together, affecting the size, monodispersity and leakage of the vesicles. Several approaches to this problem include steric stabilization with block copolymers, polymerization of either the vesicle lipids or surface-adsorbed molecules, and incorporation of cholesterol. In a novel approach, Granick *et al*²⁷ have demonstrated stabilization of liposomes by the simple incorporation of charged nanoparticles. Using 200-nanometer liposomes of 1,2-dilauroyl-sn-glycero-3-phosphocholine they added 20-nanometer carboxyl-modified polystyrene with negatively charged hydrophilic surfaces to adsorb on 25 percent of the liposome surface. This extended the stability of the liposomes against fusion for up to 50 days.

O'Brien and Liu²⁸ used cross-linking to liberate liposomes from the aqueous environment. Using the heterobifunctional lipids Acryl/DenPC_{16,18} and Sorb/DenPC_{18,20} they made large unilamellar liposomes which they subsequently polymerized. The resulting particles were freeze-dried and later rehydrated.

²⁵ Monnard, P. J. *Membr. Biol* 2003, 191, pp 87-97.

²⁶ Cisse, I.; Okumus, B.; Joo, C.; Ha, T. Fueling Protein-DNA Interactions Inside Porous Nanocontainers. *PNAS* 2006, 104(31), pp 12646-12650.

²⁷ Zhang, L.; Granick, S. How to Stabilize Phospholipid Liposomes (Using Nanoparticles). *Nanoletters* 2006, 6(4), pp 694-698.

²⁸ Liu, S.; O'Brien, D.F.; Stable Polymeric Nanoballoons: Lyophilization and Rehydration of Cross-Linked Liposomes. *JACS* 2002, 124, pp 6037-6042.

Although bilayer vesicles are generally considered to be phospholipid, this is not the only viable approach. Nakamura *et al*²⁹ have demonstrated spherical bilayer vesicles based on fullerenes. Attaching polarizable phenyl groups to fullerene provides a hydrophilic tail to the otherwise hydrophobic molecule, an example being penta-substituting fullerene with phenyl groups to form a potassium salt of pentaphenyl-fullerene. The amphiphilic nature of these constructs allowed the formation of bilayer vesicles similar to those of lipid bilayers. However these fullerene-based analogs differ from their lipid counterparts in that the fullerene “head” is the hydrophilic part while the flexible hydrocarbon “tail” associated with the surrounding solution, while lipid bilayers generally involve flexible hydrophobic tails and hydrophilic heads aligned toward the water.

Microcapsules and Polymersomes

The obvious step beyond liposomes is a rugged analog that retains the size and material transport properties of the liposome while reducing or eliminating environmental fragility and adding permeability and transport control; in other words, a microcapsule. Microencapsulation is the general term for enclosing one substance within another for a variety of purposes: to retard degradation, enhance handling properties such as powder flow, control release time, etc. For most applications microcapsules are larger than liposomes, typically in the millimeter range, and can be made by a variety of processes depending on the materials involved and the results desired. Some of the more common include pan coating, air suspension coating, coaxial jets or centrifugal extrusion, spray-drying, and polymerization. All of these have been widely applied in commercial applications ranging from common consumer products to advanced pharmaceutical and medical products, while electrified coaxial jets provide particularly good control over sub-micrometer particle size, size range, and coating thickness.³⁰

A number of encapsulation approaches have been considered in association with artificial cell and related technologies. As early as 1964, in association with his artificial cell work, Thomas Chang encapsulated 1-100 micron droplets of aqueous solutions of proteins in polymer membranes. This was achieved by interfacial polycondensation or interfacial coacervation of collodion, polystyrene and copolymers around emulsified droplets, and the result was aqueous suspensions of particulates enclosed in semipermeable microcapsules.³¹

The previous section mentioned cross-linked phospholipid bilayer vesicles, which fall into the category of micro- or nano-capsules. Discher *et al*³² have coined the term “polymersome” to describe a similar but more durable and controllable construct. Whereas phospholipid molecules have a molecular weight on the order of 750, polymers

²⁹ Zhou, S.; Burger, C.; Chu, B.; Sawamura, M.; Nagahama, N.; Toganoh, M.; Hackler, U.E.; Isobe, H.; Nakamura, E. Spherical Bilayer Vesicles of Fullerene-Based Surfactants in Water. *Science* 2001, 291, pp 1944-1947.

³⁰ Loscertales, I.G.; Barrero, A.; Guerrero, I.; Cortijo, R.; Marquez, M.; Gan<n-Calvo, A.M. Micro/Nano Encapsulation via Electrified Coaxial Liquid Jets. *Science* 2002, 295, pp 1695-1698.

³¹ Chang, T.M.S. Semipermeable Microcapsules. *Science* 1964, 146, pp 524-525.

³² http://science.nasa.gov/headlines/y2003/29may_polymersomes.htm (accessed January 2008).

can have much higher molecular weights which along with the high level of cross-linking available using polymers, allows construction of much more durable vesicles. These have been targeted for medical applications including possible dehydrated blood supplies and other artificial cell constructs.³³ The distinction between polymersomes and other durable polymer capsules is the bilayer architecture, and the amphiphilic nature of the polymers used provides properties similar to those of liposomes, with the added advantage that they are much more controllable.

In the specific case referred to in Discher's original work, polyethyleneoxide-polyethylene diblock copolymers, which self-assembled in water to form ordered lamellar phases, were formed into vesicles by an electroformation process. The resulting vesicles were 10 times less water permeable and 10 times tougher than analogous phospholipid vesicles, and are also robust and less likely than liposomes to leak encapsulated small molecules. More recently, polymersomes have been prepared by reverse evaporation techniques as well as electroformation and they are under investigation as microreaction containers.³⁴

The University of Pennsylvania team of Hammer, in collaboration with the University of Minnesota,³⁵ have used polymersomes for optical signaling by incorporating near infrared fluorophores in the lamellar membranes of polymersomes for the purposes of deep-tissue medical imaging and drug-delivery. Polymersomes were uniquely effective in uniformly distributing the fluorophores and facilitating an exceptionally intense optical signal for penetration through tissue.³⁶

Although the vast majority of research and suggested applications for polymersomes involve spherical or near-spherical vesicles, the technology isn't limited to this shape. Scientists at the National Institute of Standards and Technology have successfully used both optical tweezers and micropipettes to pull nanotubes from polymersomes³⁷, and subsequent cross-linking of the polymers stabilizes the nanotubes. These methods were capable of forming, in addition to single stand-alone nanotubes, networks of polymer nanotube nanotubes and nanotube networks connecting multiple vesicles to one another. Thus, the relatively simple concept of enclosing one material within another has expanded to the ability to connect various vesicles via controlled networks of nanotubes to net a submicron microfluidic network.

Hydrogels

Hydrogels represent a class of materials which has been employed extensively in contact lenses, medical implants, a wide variety of medical device studies dependent on tissue

³³ http://science.nasa.gov/headlines/y2003/29may_polymersomes.htm (accessed January 2008).

³⁴ www.physics.nist.gov/Divisions/Div842/Gp4/Tweezers/research.html (accessed March 2008).

³⁵ http://www.eurekalert.org/pub_releases/2005-02/uop-snp020705.php (accessed June 2008).

³⁶ Ghoroghchian, P.P.; Frail, P.R.; Susumu, K.; Blessington, D.; Branna, A.K.; Bates, F.S.; Chance, B.; Hammer, D.A.; Therien, M.J. Near-Infrared-Emissive Polymersomes: Self-Assembled Soft Matter for *in vivo* Optical Imaging. *PNAS* 2005, *102*(8), pp 2922-2927.

³⁷ Reiner, J.E.; Wells, J.M.; Kishore, R.B.; Pfefferkorn, C.; Helmerson, K. Stable and Robust Polymer nanotubes Stretched from Polymersomes. *PNAS* 2006, *103*(5), pp 1173-1177.

ingrowth, and familiar consumer products such as diaper absorbents and moisture retainers for agriculture. The term “gel” is fairly broad, referring to any dense array of cross-linked material generally with liquid filling the pores, the most common being the hydrogel, where the cross-linked material is filled with water and the material, if dehydrated, is highly absorbent. Familiar hydrogels are made from high molecular weight natural polymers such as gelatin, fibrin and polysaccharides, or high molecular weight synthetic polymers from, for example, acrylic acid, ethylene oxide, or vinyl alcohol. All hydrogels are amphiphilic, with networks of self-assembled high aspect ratio (ratio of length to width) nanofibers arranged to form high surface area hydrophilic cavities. Because hydrogels are adept at absorbing and retaining water, they are ideal materials for many biological applications. They have recently received attention from a number of sectors interested in materials bridging the biology-materials gap from the standpoint of functioning biological materials *ex-vivo* as well as the more common standpoint of using nonbiological materials in biological environments, i.e., biocompatibility.



Figure 3.2. Aerogel Supporting a Brick.

Many biological molecules such as proteins require water in order to carry out their functions. In the case of proteins this is associated with the fact that their functions generally include specific folding and unfolding sequences for which the molecules require a freedom of motion not available in the dry state. Hamachi *et al*^{38,39} addressed this problem by constructing glycosylated amino acetate hydrogels and incorporating peptide/protein arrays into the hydrophilic cavities. The environment within the cavities allows the proteins to function unhindered.

Hydrogels are being used for microenvironmental control, primarily in applications associated with encapsulated arrays of living cells. Albrecht *et al*,⁴⁰ recognizing the need for a three dimensional environment for cells in “lab-on-a-chip” style applications, encapsulated living cells in PEG-based hydrogels. They used photo-patterning to cross-link hydrogels into 100µm features and electro-patterning to position cells within these hydrogel structures with micron level resolution. Recently Schaus, Grinstaff *et al*⁴¹ have immobilized arrays of hydrogel chambers, each measuring approximately 200 microns, on glass slides for use in biological event recognition. Hydrogel chambers containing biotin, goat IgG and antisense RNA were probed with Cy5-labeled streptavidin, Cy5-labeled protein G and Cy5-labeled aRNA, respectively. These experiments successfully demonstrated recognition of small molecule-protein, protein-protein and nucleic acid-nucleic acid binding events.

³⁸ Kiyonaka, S.; Sada, K.; Yoshimura, I.; Shinkai, S.; Kato, N.; Hamachi, I. Semi-Wet Peptide/Protein Array Using Supramolecular Hydrogel. *Nature Materials* 2004, 3, pp 58-64.

³⁹ Zhang, S. Hydrogels: Wet or Let Die. *Nature Materials* 2004, 3, pp 7-8.

⁴⁰ Albrecht, D.R.; Tsang, V.L.; Sah, R.L.; Bhatia, S.N. Photo- and Electropatterning of Hydrogel-Encapsulated Living Cell Arrays. *Lab Chip* 2005, 5, pp 111-118.

⁴¹ Dominguez, M.M.; Walthier, M.; Grinstaff, M.W.; Schaus, S.E. Immobilized Hydrogels for Screening of Molecular Interactions. *Anal. Chem.* 2007, 79, pp 1064-1066.

Another area of investigation in hydrogels is gelation control, i.e., switching between viscous liquid and stable gel in response to external stimuli. Tirrell *et al*⁴² created triblock synthetic proteins, independently engineering the interchain binding and solvent retention segments of the molecules in order to allow independent investigation and control of the effects of these on gel formation, sol-gel transition temperature and reversibility. Similarly Pochan, Schneider *et al*⁴³ took advantage of the self-assembling of biomolecules to create hydrogels based on the intramolecular folding of designed peptides. The hydrogelation is itself defined by the intramolecular folding and self-assembly of monomeric β -hairpin peptides. These proteinaceous polymer gels provide additional control of responsiveness to chemical and stress environments but, unlike most protein gels, rely entirely on self-assembly in the absence of extra cross-linking agents or steps. In subsequent work the same group designed a peptide that forms a self-assembled hydrogel that is thermally responsive. The system has an amphiphilic β -hairpin that, upon heating, folds unimolecularly providing a conformation that self-assembles into a hydrogel that dissolves when temperature is again reduced.⁴⁴

The biocompatibility of hydrogels is also being exploited by the biomaterials community. Providing the possibility of controlled stiffness, swelling, degradation and molecular binding and release make them attractive materials for many applications. Alginate hydrogels, derived from a linear polysaccharide copolymer naturally occurring in algae and other marine plants, have been used in drug delivery and as extracellular matrices for basic research. In the quest for transplantable islets of Langerhans to treat Type I diabetes, cells have been entrapped in alginate hydrogels that were subsequently cross-linked with polylysine to provide permeaselectivity.⁴⁵ In further refinements of this approach, the lysine has been replaced by chitosan to improve biocompatibility.⁴⁶ In general terms, hydrogels have been effectively used in the encapsulation of living cells. Mooney and Augst⁴⁷ have demonstrated alginate hydrogels as effective scaffolds for osteoblast transplantation and improved bone formation. From a different angle on hydrogels and cells, Nakajima *et al*⁴⁸ used 1 percent strontium alginate hydrogel as an artificial cell wall, generating hydrogel beads that housed the production of invertase by protoplasts of *Saccharomyces cerevisiae*.

⁴² Petka, W.A.; Harden, J.L.; McGrath, K.P.; Wirtz, D.; Tirrell, D.A. Reversible Hydrogels from Self-Assembling Artificial Proteins. *Science* 1998, *281*, pp389-392.

⁴³ Schneider, J.P.; Pochan, D.J.; Ozbas, B.; Rajagopal, K.; Pakstis, L.; Kretsinger, J. Responsive Hydrogels from the Intramolecular Folding and Self-Assembly of a Designed Peptide. *JACS* 2002, *124*, pp13030-13037.

⁴⁴ Pochan, D.J.; Schneider, J.P.; Kretsinger, J.; Ozbas, B.; Rajagopal, K.; Haines, L.. Thermally Reversible Hydrogels via Intramolecular Folding and Consequent Self-Assembly of a *de Novo* Designed Peptide. *JACS* 2003, *125*, 11802-11803.

⁴⁵ Lim, T.; Sun, A.M. Microencapsulated Islets as a Bioartificial Endocrine Pancreas. *Science* 1908, *210*, pp 908-910.

⁴⁶ <http://www.swri.edu/3pubs/today/spring97/cell.htm> (accessed 14 May 2008).

⁴⁷ Augst, A.D.; Mooney, D.J. Alginate Hydrogels as Biomaterials. *Macromol. Biosci.* 2006, *6*(8), pp 623-633.

⁴⁸ Tanake, H.; Kamogawa, T.; Aoyagi, H.; Kato, I.; Nakajima, R. Invertase Production by *Saccharomyces cerevisiae* Protoplasts in Strontium Alginate Gel Beads. *J. Biosci. Bioeng.* 2000, *89*(5), pp 498-500.

Sidorenko *et al*⁴⁹ have focused on using hydrogel activity to control embedded materials. By controlling the stress fields within a hydrogel, achieved by humidity control, they demonstrated controlled orientation of silicon nanocolumns that had been integrated into the gel. They went on to demonstrate various complex micropatterns that could be reversibly actuated in this manner. The result is akin to a hydrogel “muscle” reacting to external stimulus.

Aerogels

Although hydrogels can be very versatile and have many applications to biologically inspired systems, by definition they are dependent on high water content and are susceptible to disablement via dehydration. This places severe limits on them in unattended environmental applications or in desert climates. A viable environmentally hardened alternative may be aerogels.

As the name implies, an aerogel can be envisioned as a hydrogel in which the water has been replaced by air. Typically, this is achieved by step-wise replacement of the water with a more volatile polar solvent, usually an alcohol to form an alcogel, and subsequent supercritical drying which removes the liquid in the absence of surface tension, eliminating the shrinkage and collapse that occurs when gels are dried under ambient conditions. This retains most of the network structure of the gel, and the result is a dry, environmentally stable material with very high surface area, very low density, low thermal conductivity (0.014 W/mK), low dielectric permittivity and unique porosity. The volume of an aerogel monolith is largely void and, in an architectural metaphor coined by Rolison and Long,⁵⁰ can be viewed as spaces that can house functions and be decorated with functional elements hung on the supporting walls and scaffolds. Along with the common silicas and aluminas, other commonly discussed metal oxide aerogels include titania, zirconia, stannic oxide and tungsten oxide. On a weight basis aerogels are very efficient and repeatable adsorbents for water, typically exceeding 1 kilogram water per kilogram aerogel for 25 cycles without degradation. However, due to the very low density this does not translate to high adsorption on a volume basis.⁵¹

The first aerogels were primarily silicas and aluminas derived from the sol-gel processes common in ceramic synthesis, and were dried in difficult high-pressure autoclave procedures. However, there are now a broad range of materials that have been used to produce aerogels and related porous materials, and recently there has been progress in alternate synthetic approaches.

One of the difficulties in synthesizing aerogels is the supercritical drying stage. During the intense investigation of aerogels in the 1980s, this generally involved cumbersome

⁴⁹ Sidorenko, A.; Krupenkin, T.; Taylor, A.; Fratzl, P.; Aizenberg, J. Reversible Switching of Hydrogel-Actuated Nanostructures Into Complex Micropatterns. *Science* 2007, 315, pp 487-490.

⁵⁰ Long, J.W.; Rolison, D.R. Architectural Design, Interior Decoration, and Three-Dimensional Plumbing En Route to Multifunctional Nanoarchitectures. *Acc.Chem.Res.* 2007, 40, pp 854-862.

⁵¹ Knez, J.; Novak, Z. Adsorption of Water Vapor on Silica, Alumina and Their Mixed Oxide Aerogels. *J.Chem.Eng.Data* 2001, 46, pp 858-860.

operations in high pressure autoclaves. More recently, critical point dryers such as those used in electron microscopy preparations have been successfully used for this purpose. In this approach the alcohol in the intermediate alcogel is replaced by liquid carbon dioxide which is removed at conditions supercritical for carbon dioxide. Others have had success with freeze-drying methods. Schiraldi *et al*⁵² have recently reported a freeze-dried clay aerogel and clay/aerogel polymer composite reinforced with natural fibers, a process which removes both the alcogel step and the super critical drying. The product exhibited compressive moduli and strengths increased significantly over non-reinforced materials, demonstrating not only vastly simplified processing techniques but also reduced fragility.

Metal aerogels are less common and are typically difficult to manufacture, with conventional approaches yielding lower surface area and larger pores than oxide aerogels. Son *et al*⁵³ have successfully made aerogel-like metal foams with density down to 0.011 grams per cubic centimeter and surface area up to 270 square meters per gram. They achieved this using self-propagating combustion synthesis of metal (Fe, Cu, Co, Ag) foams from metal complexes of bistetrazolamine (BTA).

In the search for more readily processed aerogels, Guadalupe and Guo⁵⁴ made composite gels of Bis[(3-triethoxysilyl)propyl] tetrasulfide (SIS) and sodium dodecyl sulfate (SDS). Extraction of the SDS by alcohol yielded silica aerogels with no requisite supercritical drying. The proposed mechanism suggests that the surfactant facilitates formation of SDS vesicles enclosing SIS and that the SIS polymerizes while the vesicles aggregate. Removal of the SDS by alcohol extraction produced aerogels, aided by the hydrophobicity of the polymerized SIS, indicating a novel mechanism for aerogel formation.

Another approach to simplified synthesis of aerogels is freeze-drying, an approach used in the production of SEAgel,⁵⁵ an aerogel made from agar and therefore non-toxic, biocompatible and biodegradable. The agar forms a hydrogel which, upon freeze-drying, results in SEAgel. In the related patents^{56,57} the products are called Biofoams. Biofoams are made from agar, agarose, gelatin, algin, alginates, gellan gum and cellulose which are added to a polar solvent, then either freeze-dried or emulsified with a non-polar solvent prior to gelation and freeze-drying. Composite-forming additives can be incorporated prior to gelling to modify properties of the aerogel product.

Several groups have already looked at aerogels as supports for lipid bilayers. The collaborative effort led by Stanford and UC Davis was mentioned previously. In

⁵² Finlay, K.; Gawryla, M.D.; Schiraldi, D.A. Biologically Based Fiber-Reinforced/Clay Aerogel Composites. *Ind.Eng.Chem.Res.* 2008, 47, pp 615-619.

⁵³ Tappan, B.C.; Huynh, M.H.; Hishey, M.A.; Chavez, D.E.; Luther, E.P.; Mang, J.T.; Son, S.F. Ultra-Low Density Nanostructured Metal Foams: Combustion Synthesis, Morphology and Composition. *JACS* 2006, 128, pp 6589-6594.

⁵⁴ Guo, Y.; Guadalupe, A.R. Functional Silica Aerogel from Metastable Lamellar Composite. *Chem. Commun.* 1999, pp 315-316.

⁵⁵ <http://en.wikipedia.org/wiki/SEAgel> (accessed January 2008).

⁵⁶ Morrison, R.L. Biofoam. U.S. Patent 5,382,285, January 17, 1995.

⁵⁷ Morrison, R.L. Biofoam 2. U.S. Patent 5,360,828, November 1, 1994.

association with this effort, Frank *et al*⁵⁸ used critical point dried silica aerogels from tetramethoxysilane (TMOS) and xerogels from tetraethoxysilane (TEOS) spin-coated on quartz crystal microbalance crystals as substrates for a phospholipid bilayer formed from L- α -phosphatidylcholine. The bilayer fusion on the aerogel surface was slowed significantly compared to spreading on a smooth surface. However, their experiments do indicate a continuous bilayer membrane spanning the pores in the gel and provides promise for systems in which the aerogel support provides access to both sides of the bilayer.

While the work by the Stanford group was successful in producing supported bilayers spanning the external surface of an aerogel surface, it raises the question of coating the entire aerogel surface to include internal pore surfaces. Zemanian *et al*⁵⁹ have patented a method for coating aerogel surfaces with monolayers, potentially useful for control of internal surface chemistry as well as for improving monolith strength. Schubert and Hsüsing⁶⁰ have demonstrated organofunctional aerogels from mixtures of TMOS with organofunctional alkoxysilanes, indicating the feasibility of aerogel functionalization.

Another material being investigated in aerogel form is carbon, which is typically achieved by production of an organic aerogel that is subsequently carbonized. Rodriguez-Castellon *et al*⁶¹ used resorcinol and formaldehyde for the initial organic aerogel and carbonized them at 500 or 900 degrees Celsius adding tetrabutyl orthotitanate in order to produce particulate titania inclusions. It was possible to load the aerogel with well dispersed ~4 nanometer titania particles up to a 70 percent loading. This is expected to capitalize on the synergistic effects of titania/carbon catalysts, for example for photodegradation in water purification. Other metal/carbon aerogels reported include Cu, Cr, Fe, Co, Ni, Ru, Ag, Pd and Pt, with applications in electrodes, supercapacitors, adsorbents, and catalysis.⁶² In this work they control the uniformity of the dispersion of metal dopant by replacing resorcinol with the potassium salt of 2,4-dihydroxybenzoic acid and replacing the potassium ions in the resultant gel with the desired metal ion, in this particular case Cu.

Another interesting family of materials is aerogel composites, most often with nanotubes. Kaneko *et al*⁶³ embedded single-wall carbon nanohorns into resorcinol-formaldehyde organic aerogels. Since the nanohorns were embedded in the aerogel after it was dried and the gel was not carbonized, the properties of the nanohorns remain intact and the

⁵⁸ Weng, K.C.; StAlgren, J.J.R.; Duval, D.J.; Risbud, S.H.; Frank, C.W. Fluid Biomembranes Supported on Nanoporous Aerogel/Xerogel Substrates. *Langmuir* 2004, 20, pp 7232-7239.

⁵⁹ Zemanian, T.S.; Fryvelli, G.; Ustyugov, O.A. Monolayer Coated Aerogels and Method of Forming. U.S. Patent 7,019,037, March 28, 2006.

⁶⁰ Husing, N.; Schubert, U. Organofunctional Silica Aerogels. *J.Sol-Gel Sci.Tech.* 1997, 8, pp 807-812.

⁶¹ Morena-Castilla, C.; Maldonado-Huñdar, F.J.; Carrasco-Marín, F.; Rodríguez-Castellón, E. Surface Characteristics of Titania/Carbon Composite Aerogels. *Langmuir* 2002, 18, pp 2295-2299.

⁶² Fu, R.; Yoshizawa, N.; Dresselhaus, M.S.; Dresselhaus, G.; Satcher, J.H.Jr.; Bauman, T.F. XPS Study of Copper Doped Carbon Aerogels. *Langmuir* 2002, 18, pp 10100-10104.

⁶³ Tao, Y.; Noguchi, D.; Yang, C.-M.; Kanoh, H.; Tanaka, H.; Yudasaka, M.; Iijima, S.; Kaneko, K. Conductive Mesoporous Single-Wall Carbon Nanohorn/Organic Aerogel Composites. *Langmuir* 2007, 23, pp 9155-9157.

resulting composite materials showed high electrical conductivity and controllability of pore sizes.

There has been very little investigation of aerogel systems based on chalcogenides; however the work of Kanatzidis *et al*⁶⁴ demonstrates that such systems are possible and that they combine the electronic properties of the chalcogenides with the internal porosity of aerogels. They developed a strategy for forming semiconducting aerogels derived from chalcogenide clusters with platinum linking ions. They prepared chalcogels with the formula $Pt_2[M_4Q_{10}]$ where $M = Ge, Sn$ and $Q = S, Se$. These materials are stable in humid environments, have high affinity for hydrophobic species, and absorb light in the visible and infrared.

In a novel application of aerogel technology, Risen *et al*⁶⁵ have generated hybrid aerogels of bioderived polymers and silica, working with chitosan, pectic acid and alginic acid. Their purpose has been to subsequently react the biopolymers to form new materials which can be harvested by dissolution of the aerogel scaffold. This work is mentioned here as it relates to chitosan, a high interest material in the ANTS program.

⁶⁴ Bag, S.; Trikalitis, P.N.; Chupas, P.J.; Armatas, G.S.; Kanatzidis, M.G. Porous Semiconducting Gels and Aerogels From Chalcogenide Clusters. *Science* 2007, 317, pp 490-493.

⁶⁵ Wang, M.; Liu, X.; Ji, S.; Risen, W.M.Jr. A New Hybrid Aerogel Approach to Modification of Bioderived Polymers for Materials Applications. In *Materials Research Society Symposium Proceedings* 2002, 702, pp 77-85.

Table 2. Comparison of aerogel and related high surface area solids

	Density (g/cc)	Surface area (m ² /g)	Pore sizes
Traditional oxide aerogel	0.003-0.35	500-1000	<100 nm
Clay	0.07		
Reinforced clay/composite			
Typical metal foam (Al, Mg)	0.04-0.08		
Self-propagating combustion Metal Foam	0.011	270	1-3 μ m 20-200 nm
Silica/polymer hybrid	0.357	766	11.5 nm
Biofoam/SEAgel	0.0012-0.5 Typical 0.3	10	2-3 μ m
Chalcogel	0.12-0.17	117-327	

Porous Inorganics

Nanoporous materials are not limited to aerogels and a number of relevant studies exist regarding nanoporous inorganic materials in biomimicry. Nanoporous alumina membranes from anodized aluminum have been used to imitate ion diffusion and ion pumping using experimental techniques similar to those used with bilipid membranes.⁶⁶ Based on the honeycomb morphology of anodic alumina, Fukuda *et al*⁶⁷ have used anodic porous alumina as a template for the production of morphological replicas in platinum and gold.

Functionalized mesoporous silica has been used for entrapment of enzymes such that the kinetics of entrapped enzymes approached reaction rates in solution.⁶⁸ In a related study, enzymes entrapped in functionalized mesoporous silica have been observed to retain activity that would have been lost in the absence of the mesoporous surroundings,

⁶⁶ Wang, G.-J.; Chang, W.C. Imitation of the Ion-Diffusion and Ion Pumping Mechanism of a Living Cell by Nanoporous Alumina Membrane. In *ENS '05*, 14-16 December 2005, Paris, France.

⁶⁷ Masuda, H.; Fukuda, K. Ordered Metal Nanohole Arrays Made by a Two-Step Replication of Honeycomb Structures of Anodic Alumina. *Science* 1995, 268, pp 1466-1468.

⁶⁸ Lei, C.; Shin, Y.; Magnuson, J.K.; Fryxell, G. Lasure, L.L.; Elliott, D.C.; Liu, J.; Ackerman, E.J. Characterization of Functionalized Nanoporous Supports for Protein Confinement. *Nanotech.* 2006, 17, pp 5531-5538.

hypothetically because the crowding within the pores forces protein folding.⁶⁹ As mentioned previously, Nishiya *et al.*,⁴ among others, used porous inorganics as substrates for lipid bilayers in biosensing. Lopez *et al.*⁷⁰ extended this to Mesoporous silica microbeads, which they used for bilipid supports for use with transmembrane proteins, while scientists at the Paul Scherrer Institut have found that BLMs on ultrathin nanoporous silicon nitride retain fluidity but not stability.⁷

In a novel twist on the idea of nanopores, Achim Muller *et al* have been studying polyoxomolybdate clusters.^{71,72} Placing protonated urea ions in the pores provides a molecular stopper that can be opened to allow ion uptake and subsequently closed. Although this specific technology may not be immediately relevant, the ability to build inorganic molecular constructs that can controllably behave in a manner homologous to a biological functional element is revolutionary. The possibility of using a single stoppered pore as an abiotic channel in an abiotic membrane remains to be investigated.

Chitosan

Another material of high interest due to its inherent biocompatibility is chitosan. Chitin, the precursor to chitosan, is a naturally occurring biopolymer, a polysaccharide found prevalently in the exoskeletons of arthropods, including crustaceans. While chitin is a partially deacetylated *N*-acetylglucosamine, chitosan is a more extensively deacetylated form of the same material. Chitosan is commercially available in a range of degrees of deacetylation, typically between 60 and 100 percent, and molecular weights. Alternately, it can be produced by enzymatic deacetylation of chitin from a number of natural sources. It is positively charged and soluble in acidic solutions.

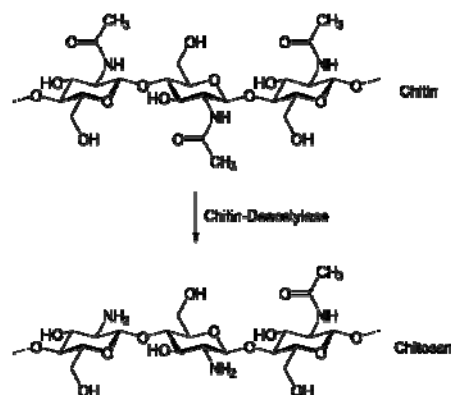


Figure 3.3. Chitosan from Deacetylation of Chitin.

Commercial uses for chitosan are diverse. In the biomedical field it has been found to aid blood clotting and is used in field bandages; in agriculture it is used to enhance growth and protect against fungal infestations; in filtration and brewing it is used to scavenge sediment particles; in the weight loss industry it is sold with claims that it removes dietary fat; and claims have been made that it can reduce LDL cholesterol and increase HDL cholesterol. Recently, the properties of chitosan have drawn attention in the field of

⁶⁹ <http://www.pnl.gov/topstory.asp?id=202> (accessed June 2008)

⁷⁰ Buranda, T.; Huang, J.; Ramaro, G.V.; Ista, L.K.; Larson, R.S.; Ward, T.L.; Sklar, L.A.; Lopez, G.P. Biomimetic Molecular Assemblies on Glass and Mesoporous Silica Microbeads for Biotechnology. *Langmuir* 2003, 19, pp 1654-1663.

⁷¹ Freemantle, M. Artificial Cells Allow Ion Entry. *Chemical and Engineering News*, 2005, 83(48), pg 10.

⁷² www.physorg.com/printnews.php?newsid=8079 (accessed June 2008)

biofabrication for a variety of applications⁷³ based on its film forming and electronic properties which allow for directed assembly and ease of biological functionalization.

For purposes of protein immobilization, the acid environment of chitosan solution is harmful to the proteins while calcium alginate hydrogels, often used in the immobilization of enzymes, are damaged by phosphate ion in physiological buffer. Amiji *et al*⁷⁴ addressed this with hybridized chitosan-alginate microcapsules. They prepared layered microcapsules of chitosan over alginate, adding enzyme to the process step in which the alginate cores are generated. This yielded enzyme-bearing alginate cores protected by outer layers of chitosan. The chitosan layer provided permeability selectivity and protection while the alginate protected the enzyme from the harsh environment of chitosan solution, with the overall structure stabilizing and extending the activity of the enzyme.

There are many ways to fabricate chitosan microspheres for drug delivery. Sinha *et al*⁷⁵ compared several of these with respect to their mucoadhesive properties, which are related to electrical charge. In general, chitosan carries a net positive charge which impacts its interactions with biological systems. Sinha's work indicates from zeta potential measurements that the charge is strongly affected by the production method chosen, as is stability at low pH.

Groups at Southwest Research Institute and the University of Pittsburgh Medical Center have been developing methods to encapsulate insulin-producing cells for transplantation to fight diabetes. Their efforts led to alginate hydrogels cross-linked with polylysine. However, this faces immunorejection problems, and chitosan has been used as a replacement for the lysine, allowing for the selective permeability of the original materials without the immunorejection problems of the lysine.

As a biocompatible and non-toxic material with antimicrobial properties, chitosan has also received attention for applications in the food industry. With food applications in mind, Kim *et al*⁷⁶ and Nadarajah *et al*⁷⁷ have studied the effect of pH and solvent on the properties of chitosan films, specifically water vapor permeability, tensile strength and elongation. In both studies the acid system chosen (formic, lactic, acetic, propionic, ascorbic, malic) had significant effect on the product, particularly in terms of strength, flexibility, hydrophilicity, and appearance.

⁷³ Yi, H.; Wu, L.-Q.; Bentley, W.E.; Ghodssi, R.; Rubloff, G.; Culver, J.N.; Payne, G. Biofabrication with Chitosan. *Biomacromolecules* 2005, 6(6), pp 2881-2894.

⁷⁴ Taqieddin, E.; Lee, C.; Amiji, M. Perm-Selective Chitosan-Alginate Hybrid Microcapsules for Enzyme Immobilization Technology. *Pharmaceutical Engineering* 2002, 22(6), pp 1-3.

⁷⁵ Dhawan, S.; Singla, A.K.; Sinha, V.R. Evaluation of Mucoadhesive Properties of Chitosan Microspheres Prepared by Different Methods.

⁷⁶ Kim, K.M.; Weller, C.L.; Hanna, M.A. Properties of Chitosan Films According to pH and Types of Solvents. [Online] *IFT Annual Meeting*, 12-16 July 2004, Las Vegas, NV, Session 83C.

⁷⁷ Nadarajah, K.; Prinyawiwatkul, W.; No, H.K. Physicochemical Properties of Chitosan Edible Films Developed From Crawfish Shell Waste. [Online] *IFT Annual Meeting*, 15-20 July 2005, New Orleans, LA.

Another area that has received a great deal of attention is composites of chitosan and carbon nanotubes. Tong *et al*⁷⁸ used simple solution-evaporation to prepare composites of multiwalled carbon nanotubes in chitosan. Distribution of nanotube was found to be homogeneous even with this simple process and other reports⁷⁹ have suggested that this can be attributed to the polymer chains wrapping around the individual nanotubes. With very low (<1 percent) nanotube loading the tensile modulus was improved by 93 percent and strength increased by 99 percent. Chitosan facilitates the stabilization of carbon nanotubes, a property exploited by Gorski *et al*⁷⁹ to deposit chitosan-carbon nanotube composite films on glassy carbon electrodes. They subsequently immobilized enzyme in the film, another process facilitated by chitosan and the resulting electrodes were successfully used as glucose sensors. This approach should be readily adaptable to other analytes. Chen *et al*⁸⁰ used a similar philosophy to fabricate chitosan-carbon nanotube composite films on gold electrodes. In this work the film was electrodeposited, capitalizing on the pH dependence of chitosan solubility. This device was also demonstrated as a glucose sensor. Ruzgas *et al*⁸¹ used single wall carbon nanotubes (SWCNT) in chitosan to form films on glassy carbon electrodes. In this case the films were further stabilized by cross-linking with glutaraldehyde which also presented free aldehyde groups for further functionalization. Immobilizing galactose oxidase on these surfaces provided an effective electrochemical galactose sensor.

The biocompatibility and fabrication versatility of chitosan make it an attractive material for a range of biological and biomimetic applications. In addition to the investigations mentioned here, chitosan is being considered for use in aerogel-related materials,⁶⁵ in compositions with collagen for piezoelectric properties,⁸² in water-soluble derivatives for improved properties for biological applications,⁸³ and in fabric treatment schemes to improve properties and instill antimicrobial capabilities.⁸⁴

S-layer Proteins

S-layer proteins are natural proteins originating from the surface layer of bacterial cells and archaea. They are crystalline and have the ability to self-assemble into two-dimensional arrays. They can be lithographically patterned and have drawn interest because, in addition to patterning, they can act as templates for the deposition of

⁷⁸ Wang, S.-F.; Shen, L.; Zhang, W.-D.; Tong, Y.-J. Preparation and Mechanical Properties of Chitosan/Carbon Nanotube Composites. *Biomacromol.* 2005, 6(6), pp 3067-3072.

⁷⁹ Zhang, M.; Smith, A.; Gorski, W. Carbon nanotube-Chitosan System for Electrochemical Sensing Based on Dehydrogenase Enzymes. *Anal.Chem.* 2004, 76(17), pp 5045-5050.

⁸⁰ Luo, X.-L.; Xu, J.-J.; Wang, J.-L.; Chen, H.-Y. Electrochemically Deposited Nanocomposite of Chitosan and Carbon Nanotubes for Biosensor Application. *Chem. Commun.* 2005, pp 2169-2171.

⁸¹ Luo, X.-L.; Xu, J.-J.; Wang, J.-L.; Chen, H.-Y. Electrochemically Deposited Nanocomposite of Chitosan and Carbon Nanotubes for Biosensor Application. *Chem. Commun.* 2005, pp 2169-2171.

⁸² Silva, C.C.; Lima, C.G.; Pinheiro, A.G.; Gomes, J.C.; Figueiredo, S.D.; Sombra, A.S.B. On the Piezoelectricity of Collagen-Chitosan Films. *Phys. Chem. Chem. Phys.* 2001, 3, 4154-4157.

⁸³ Cho, J.; Grant, J.; Piquette-Miller, M.; Allen, C. Synthesis and Physicochemical and Dynamic Mechanical Properties of a Water-Soluble Chitosan Derivative as a Biomaterial. *Biomacromol.* 2006, 7, pp 2845-2855.

⁸⁴ Jeong, Y.J.; Cha, S.Y.; Yu, W.R.; Park, W.H. Changes in the Mechanical Properties of Chitosan-Treated Wool Fabric. *Tex. Res. J.* 2002, 72(1), pp 71-76.

inorganic moieties with electronic and/or optical properties. For these reasons they are considered important components for “biomolecular construction kits.”⁸⁵

S-layer protein lattices are disrupted by chaotropic agents, pH control, or metal chelating agents, after which they reassemble upon removal of the disrupting agent. Typically, reassembly can take the form of sheets, cylinders or vesicles with two-dimensional lattice parameters characteristic of the specific protein, being square, hexagonal or oblique. S-layer thicknesses are on the order of 4 to 10 nanometers and lateral domain measurements range from nanometers to many microns, with morphology dependent on environmental factors, especially temperature, pH and ionic composition and strength.

One of the important features of S-layer proteins is their ability to self-assemble into these arrays at a variety of interfaces. Upon removal of the disrupting agent, S-layers will readily form at liquid-gas or liquid-solid interfaces. These assembled arrays are molecular monolayers with well-defined two dimensional crystal symmetries. Formation of S-layers at solid-liquid interfaces provides a valuable tool for surface preparation for subsequent functionalization, and surfaces that have been reported as substrates for S-layers include Si with a native oxide layer, silanized silicon wafers, and silicon coated with carbon or photoresist, as well as silicon nitride, gallium arsenide, glass, polymers, noble metals, mica and graphite. The substrate surfaces are typically hydrophobic, hence the surface preparations for silicon substrates, although some proteins have been found to assemble on hydrophilic surfaces as well.⁸⁵ For assembly on solid surfaces, the coherence and domain size of the layer depend on the substrate surface as well as the identity and environment of the protein. One relevant application of S-layers on solid substrates is the stabilization of supported lipid membranes. Lipids on S-layers are both more fluid and more stable than lipids supported in other ways. This is significant in applications associated with pore-forming proteins, which tend to rupture lipid membranes that are not supported on S-layers. Sleytr *et al*⁸⁶ demonstrated the enhanced bilayer membrane-stabilizing effect of S-layer lattices over microfiltration membranes as well as reconstitution of functional α HL pores within these S-layer ultrafiltration membranes.

At the liquid-air interface S-layers assemble as monolayers on the liquid, typically water, surface. The morphology of the monolayer is highly dependent on ion content in the solution, with a 10 millimolar concentration of bivalent cation being optimum for long-range order. This suggests a salt bridge mechanism for stabilizing coherent layers.

Patterning is often required for practical applications. S-layers are easily patterned lithographically using photomasking and deep ultraviolet radiation. Combining this with functionalization allows precise positioning of a range of molecules on the nanometer scale. In the case of macromolecules, this can be viewed as preferential deposition on the S-layer molecules resulting in patterning of the functional species that mimics the

⁸⁵ Pum, D.; Neubauer, A.; Gyoervary, E.; S<ra, M.; Sleytr, U.B. S-Layer Proteins as Building Blocks in a Biomolecular Construction Kit. Presented at the 7th Foresight Conference on Molecular Nanotechnology and accessed online <http://www.foresight.org/conference/MNT7/Papers/Pum/index.html>.

⁸⁶ Schuster, B.; Pum, D.; S<ra, M.; Braha, O.; Bayley, H.; Sleytr, U.B. S-layer Ultra-Filtration Membranes: A New Support for Stabilizing Functionalized Lipid Membranes. *Langmuir* 2001, 17, pp 499-503.

underlying pattern of the protein layer. Smaller inorganic molecules the S-layer can act as a mask, with precipitation of the inorganic species occurring in the pores of the S-layer to produce an array of nanoparticles with well defined symmetry. Batt *et al*⁸⁷ determined that this biotemplating is dependent on interactions between the growing nanoparticles as well as on interactions between the S-layer and the growing particles. They used a hexagonally packed intermediate layer of *Dinococcus radiodurans*, a hexameric structure with pores at the centers of the hexamer units and large openings at the vertices visualized as the corners of the hexagonal units, used to template ordered arrays of citrate-capped gold nanoparticles. Nanoparticle binding at the vertices was found to be controlled by ionic strength, with low ionic strength allowing ionic repulsions leading to nanoparticle deposition on alternating vertices while higher ionic strength lowered the effects of interparticle repulsion and allowed deposition on each vertex. Mark *et al*⁸⁸ extended this work to use both *Deinococcus radiodurans* and *Sulfolobus acidocaldarius* S-layers to template citrate-capped gold and thiol-functionalized CdSe/ZnS quantum dots to control the patterning of the nanoparticle arrays.

Batt *et al*⁸⁹ have recently demonstrated the ion transport properties of S-layers via electrical impedance spectroscopy. Their work indicates that calcium ions are transported through the vertices of the lattice, with transport controlled primarily by electrical gradients. Thus, while it is possible⁸⁵ to insert ion channel pores into bilayers and S-layers, using the pore structures inherent in the S-layer may also be a viable route to biologically based nanofiltration and sensing.

Molecular Imprints

Molecular imprinting is the practice of synthesizing an abiotic material which is morphologically equivalent to a biological functional element whose function is connected to morphology. In simplistic terms, an example might be a polymer that has been molded around an antigen so that, on removal of the antigen, the polymer takes on the antibody morphology and can act as an artificial antigen capture agent. The selectivity of this approach was demonstrated by Valdes, Thompson, *et al* in a study in which they used molecularly imprinted polymers (MIPs) to selectively capture closely related conotoxins.⁹⁰ In practical terms, using the analyte as the imprint template, although direct and effective, is not ideal as it introduces analyte to the sensor production process and resulting MIPs may be self-contaminating, a condition referred to as template bleeding.

⁸⁷ Bergkvist, M.; Mark, S.S.; Yang, X.; Angert, E.R.; Batt, C.A. Bionanofabrication of Ordered Nanoparticle Arrays: Effect of Particle Properties and Adsorption Conditions. *J.Phys.Chem.B.* 2004, *108*, pp 8241-8248.

⁸⁸ Mark, S.; Bergkvist, M.; Yang, X.; Teixeira, L.M.; Bhatnagar, P.; Angert, E.R.; Batt, C.A. Bionanofabrication of Metallic and Semiconductor Nanoparticle Arrays using S-Layer Protein Lattices with Different Lateral Spacings and Geometries. *Langmuir* 2006, *22*, pp 3763-3774.

⁸⁹ Sotiropoulou, S.; Mark, S.S.; Angst, E.R.; Batt, C.A. Nanoporous S-Layer Protein lattices: A Biological Ion Gate with Calcium Selectivity. *J.Phys.Chem.C.* 2007, *111*, pp 13232-13237.

⁹⁰ Iqbal, S.S., Lulka, M.F., Chambers, J.P., Thompson, R.G., Valdes, J.J. *Materials Science & Engineering C-Biomimetic And Supramolecular Systems*, 2000, *7*, pp77-81.

In chromatography, MIPs have received a great deal of interest for their ability to selectively separate specific molecules from solution and they have proven quite useful in this capacity.⁹¹ In the field of drug delivery, MIPs have been considered favorably for controlled and sustained release, enantio-selective loading and release and improved loading.⁹² In the biological arena, MIPs are of interest for a number of applications. Bolisay *et al*⁹³ have been working on molecularly imprinted polymers for the recognition, differentiation and, ultimately, removal of viruses. In contrast with typical MIP synthesis strategies that involve monomers in organic solutions, they use polyamine cross-linked in aqueous solutions. Their poly(allylamine hydrochloride) hydrogels imprinted against baculovirus appear to be effective at removing baculovirus from solution, a result that is very significant for its prospects in removal of viruses for a broad range of requirements. Another important discovery in this work is the relative insensitivity of the hydrogel MIP to swelling effects, long considered a problem in monomer-derived MIPs but apparently not significant with the polymer synthesis approach. Chen *et al*⁹⁴ and Waigmann *et al*⁹⁵ have reported surface imprinted polymers used to detect low concentrations of virus, while Bolisay *et al*⁹⁶ work with three dimensional polymer gels for collection of large amounts of virus. In this recent work they have demonstrated the shape selectivity of MIPs imprinted for virus binding.

Several approaches have been considered to overcome the issue of template bleeding. Sellergren⁹⁷ and Takeuchi⁹⁸ developed combinatorial methods for synthesizing and screening MIPs for specific targets while Piletsky *et al*⁹⁹ use modeling for the same purpose. While these approaches address the problem from the aspect of avoiding the target analyte in MIP synthesis, Chang *et al*¹⁰⁰ approach the problem from the template release aspect. They used a thermally reversible bond for imprinting silica spheres while introducing functional groups into the cavity vacated by the template.

Molecularly imprinted polymers have been investigated as sensors in a range of schemes. They have been used on electrodes for electrochemical sensing,^{101, 102} on quartz crystal

⁹¹ Widstrand, C.; Yilmaz, E.; Boyd, B.; Rees, A. *the Column* [Online] 2006, Feb., pp 20-24.

⁹² Hilt, J.Z.; Byrne, M.E. Configurational Biomimesis in Drug Delivery: Molecular Imprinting of Biologically Significant Molecules. *Adv. Drug Deliv. Rev.* 2004, 56(11), pp 286-315.

⁹³ Bolisay, L.D.V.; March, J.F.; Bentley, W.E.; Kofinas, P. Separation of Baculoviruses Using Molecularly Imprinted Polymer Hydrogels. In *Mat. Res. Soc. Symp. Proc. Vol 787*, G3.1.1-G.3.1.5.

⁹⁴ Tai, D.F.; Lin, C.Y.; Wu, T.Z.; Chen, L.K. Recognition of Dengue Virus protein Using Epitope-Mediated Molecularly Imprinted Film. *Anal. Chem.* 2005, 77(16), pp 5140-5143.

⁹⁵ Dickert, F.L.; Hayden, O.; Bindeus, R.; Mann, K.J.; Blaas, D.; Waigmann, E. Bioimprinted QCM Sensors for Virus Detection – Screening of Plant Sap. *Anal. Bioanal. Chem.* 2004, 378(8), pp 1929-34.

⁹⁶ Bolisay, L.D.; Culver, J.N.; Kofinas, P. Molecularly Imprinted Polymers for Tobacco Mosaic Virus Recognition. *Biomaterials* 2006, 27, pp 4165-4168.

⁹⁷ Lanza, F.; Sellergren, B. Method for Synthesis and Screening of Large Groups of Molecularly Imprinted Polymers. *Anal. Chem.* 1999, 71, pp 2092-2096.

⁹⁸ Takeuchi, T.; Fukuma, D.; Matsui, J. *Anal. Chem.* 1999, 71, pp 285-290.

⁹⁹ Piletsky, S.A.; Piletska, E.V.; Day, C.J.; Freebairn, K.W.; Legge, C.; Turner, A.P.F. *Analyst* 2001, 126, pp 1826-1830.

¹⁰⁰ Ki, C.D.; Oh, C.; Oh, S.-G.; Change, J.Y. The Use of a Thermally Reversible Bond for Molecular Imprinting of Silica Spheres. *J. Am. Chem. Soc.* 2002, 124, pp 14838-14839.

¹⁰¹ Shoji, R.; Takeuchi, T.; Kubo, I. Atrazine Sensor Based on Molecularly Imprinted Polymer-Modified Gold Electrode. *Anal. Chem.* 2003, 75(18), pp 4882-4886.

microbalances for gravimetric sensing¹⁰³ including whole cell applications,¹⁰⁴ and in arrays for colorimetric identification.¹⁰⁵

In addition to bulk and thin film morphologies and porous monoliths, there is a growing body of work applying imprinting techniques to nanoparticles. For example, Mayes *et al*¹⁰⁶ have demonstrated the binding of propanolol by shell-imprints on core-shell nanoparticles and report the effect of solvent on binding affinity, while Zhu *et al*¹⁰⁷ have devised composites of multiwalled carbon nanotubes with MIPs to join the electronic properties of nanotubes with the recognition capabilities of the MIPs.

Nanotubes

No discussion of emerging materials is complete without touching on nanotubes. Although nanotubes are not relevant to the current discussion as stand-alone substrates for abiotic sensing applications they are extremely interesting as composite elements in the substrates, and have in fact been mentioned previously for cases where the composites are already under investigation.

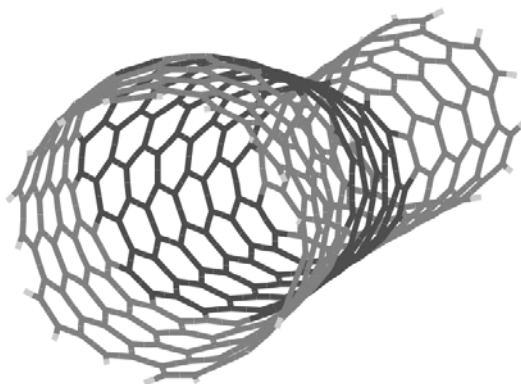


Figure 3.4. Conceptual Drawing of a Single-Walled Carbon Nanotube

By far the most commonly studied nanotubes are carbon nanotubes, which come in both the single walled (SWCNT) and multiwalled (MWCNT) varieties. SMCNTs can be envisioned as single planar sheets of graphite, graphene, wrapped into tubes with diameters on the order of a nanometer and lengths up to the micron range. They have excellent electrical properties and can be modified to make both p- and n- field effect transistors and therefore can be used to construct nanoscale logic gates.¹⁰⁸ Generally the electrical properties of SWCNTs are dictated by the wrapping symmetry of the tube, which in turn dictates the available electron paths. Controlling these properties is at the heart of SWCNT applications in nanoelectronics.

¹⁰² Kitada, T.; Kitamura, K.; Konishi, T.; Takegami, S.; Okuno, T.; Ishikawa, M.; Wakabayashi, M.; Nishikawa, K.; Muramatsu, Y. Potentiometric Immunosensor Using Artificial Antibody based on Molecularly Imprinted Polymers. *Anal. Chem.* 2004, 72(22), pp 6802-6807.

¹⁰³ Das, K.; Penelle, J.; Rotello, V.M. Selective Picomolar Detection of Hexachlorobenzene in Water Using a Quartz Crystal Microbalance Coated with a Molecularly Imprinted Polymer Thin Film. *Langmuir* 2003, 19(9), pp 3921-3925.

¹⁰⁴ Dickert, F.L.; Hayden, O. *Anal. Chem.* 2002, 74, pp 1302-1306

¹⁰⁵ Buryak, A.; Severin, K. A. Chemosensor Array for the Colorimetric Identification of 20 Natural Amino Acids. *J. Am. Chem. Soc.* 2005, 127(11), pp 3700-3701.

¹⁰⁶ Perez-Moral, N.; Mayes, A.G. Noncovalent Imprinting in the Shell of Core-Shell Nanoparticles. *Langmuir* 2004, 20, pp 3775-3779.

¹⁰⁷ Kan, X.; Zhao, Y.; Geng, Z.; Wang, Z. Zhu, J.-J. Composites of Multiwalled Carbon nanotubes and Molecularly Imprinted Polymers for Dopamine Recognition. *J. Phys. Chem. C.* 2008, 112, pp 4849-4854.

¹⁰⁸ Martel, R.; Derycke, V.; LaVoie, C.; Appenzeller, J.; Chan, K.K.; Tersoff, J.; Avouris, Ph. Ambipolar Electrical Transport in Semiconducting Single-Wall Carbon Nanotubes. *Phys. Rev. Lett.* 2001, 87(25), pp 256805.

The drawbacks of SWCNTs are the synthetic expense and susceptibility to chemicals. A special case of the multiwalled nanotube, the double walled nanotube, is significantly less susceptible to chemicals but remains difficult and expensive to manufacture.

Multiple layers of graphene wrapped into a tube constitute a MWCNT. In comparison with SWCNTs, multiwalled tubes sacrifice electrical properties in favor of stability and ease of manufacture. MWCNTs are larger than SWCNTs, with outer diameters ranging from >1 to 100 nanometers and variable inner diameters. In many applications where the electrical properties of SWCNTs are not required, these less expensive relatives are appropriate.

Composites of multiwalled carbon nanotubes in chitosan have already been mentioned⁷⁸ and these composites have been cast⁷⁹ and electrochemically deposited⁸¹ on electrodes for biosensing applications. SWCNTs have likewise been used in chitosan composites on glassy carbon electrodes for galactose sensing.⁸¹

Another potential value of carbon nanotubes is their ability to cross cell membranes. They can be readily functionalized and used to ferry functionality into a cell.¹⁰⁹ This property can be employed to move functional elements across abiotic membranes. Additionally it is possible to incorporate nanotubes into membranes, with the nanotubes acting as pores. In microfluidics it has been found that the hydrophobicity and smooth inner surface of carbon nanotubes accelerates the motion of fluids through nanotubes.¹¹⁰ This concept has been used in aligning MWCNTs in polymer membranes used to demonstrate molecular transport through the tubes.¹¹¹ Functionalization of the inner surfaces of the CNTs is also possible. Similar to the enhanced flow through CNTs, the concentric layers of MWCNTs move freely across each other, allowing telescoping action and free rotation which has been demonstrated in molecular machines.

Although carbon nanotubes are by far the most heavily studied nanotubes, they are by no means the only players in the game. Tenne *et al*¹¹² have made layered tubes of sulfides and selenides of tungsten and molybdenum some of which are used commercially as solid lubricants. Yang *et al*¹¹³ recognized the suitability of nanowires as templates for the growth of nonlayered nanotubes of gallium nitride and silica which have subsequently been used to demonstrate regulation of ionic transport. Arrays of titania nanotubes from

¹⁰⁹ Pantarotto, D.; Briand, J.-P.; Prato, M.; Bianco, A. Translocation of Bioactive peptides Across Cell Membranes by Carbon Nanotubes. *Chem. Commun.* 2004, pp 16-17.

¹¹⁰ Holt, J.K.; Park, H.G.; Wang, Y.; Stadermann, M.; Artyukhin, A.B.; Grigoropoulos, C.P.; Noy, A.; Bakajin, O. Fast Mass Transport through Sub-2-Nanometer Carbon Nanotubes. *Science* 2006, *312*, pp 1034-1037.

¹¹¹ Scholl, D.S.; Johnson, J.K. Making High-Flux Membranes with Carbon nanotubes. *Science* 2006, *312*, pp 1003-1004.

¹¹² Rapoport, L.; Fleischer, N.; Tenne, R. Applications of WS₂ (MoS₂) Inorganic Nanotubes and Fullerene-Like Nanoparticles for Solid Lubrication and for Structural nanocomposites. *J. Mater. Chem.* 2005, *15*, pp 1782-1788.

¹¹³ Fan, R.; Yue, M.; Karnik, R.; Majumdar, A.; Yang, P. Polarity Switching and Transient Responses in Single Nanotube Nanofluidic Transistors. *Phys. Rev. Lett.* 2005, *95*, 086607.

anodization of titanium electrodes by Grimes *et al*¹¹⁴ are more sensitive hydrogen detectors than any other technology investigated, perhaps as the result of the nanotubes and inter-tube spaces providing a well defined straight path at the molecular level.

A broad array of inorganic nanotubes have been synthesized and studied, both singly and in arrays, and a full review of these is not appropriate here. The salient point is that nanotube applications need not be limited by the fairly narrow array of sizes and properties available from carbon allotropes. A much broader and more controllable array of properties and morphologies is available by considering materials other than carbon.

In the context of designing adaptive abiotic materials and systems, SWCNTs may be of interest as embedded electrically active subunits but not as an essential part of the substrate or matrix material. MWCNTs on the other hand may be useful in the substrate as structural composite reinforcements or as synthetic pores or conduits embedded into the matrix.

¹¹⁴ Varghese, O.K.; Mor, G.K.; Grimes, C.A.; Paulose, M.; Mukherjeeb, N. A Titania Nanotube-Array Room-Temperature Sensor for Selective Detection of hydrogen at low Concentrations. *J. Nanosci. Nanotech.* 2004, 4, pp733-737.

Conclusions and Recommendations

The purpose of this review was to survey the field of biologically inspired materials and to develop a conceptual path from natural, biotic materials to completely synthetic, abiotic materials which can be assembled into systems with unique properties found in living systems but which could not be replicated via traditional synthetic chemical technologies. The ability to design and manufacture abiotic components which can be assembled into adaptive systems will have applications ubiquitous to the defense industry. Examples include sensors, adaptive camouflage, antibiotic and antiviral agents, compact personal power, coatings, new green manufacturing processes, the list is endless. As a specific example, the ANTS platform concept is a new paradigm in the area of CBD and is the result of a convergence of a number of rapidly developing technical areas, including synthetic biology, nanotechnology, materials science and information technology. This “transdisciplinary” approach is inimicable to current DOD research practices but is quickly becoming the norm in the commercial and academic sectors. Just as the current administration has placed an emphasis on radically altering acquisition policy and practice, so too must the DOD research community embrace radical change and develop both a philosophy and processes which facilitate transdisciplinary research and development.